

The University of Chicago

EXTRACTION OF AUXIN
FROM MAIZE, FROM SMUT TUMORS
OF MAIZE, AND FROM
USTILAGO ZEAE

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1941

By
JAMES E. MOULTON

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EXTRACTION OF AUXIN FROM MAIZE, FROM SMUT TUMORS OF MAIZE, AND FROM *USTILAGO ZEA*¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 541

JAMES E. MOULTON

Introduction

This investigation was undertaken to test the hypothesis that if growth-affecting or growth-affecting substances play an important role in the normal growth of plants, then the growth disturbances observed in pathological conditions, such as in the corn-smut tumors being studied, should be correlated with disturbed auxone relations in the infected plants. Although many substances are classified as auxones, only those (the auxins) which can be detected by the standard *Avena* coleoptile technique have been studied here. It is not assumed, however, that auxins thus determined are the sole or primary causes of the responses incited by the pathogen, *Ustilago zea*, but rather that they may be among the compounds correlated with the abnormal activity of the diseased tissues.

Early in this investigation it became apparent that the current procedures for the extraction of auxin from plant material were not adequate, for various reasons. Work done in this laboratory and elsewhere has shown that quantitative auxin extraction from green tissues is not easily accomplished (5, 10, 12, 20). As yet no adequate technique has been found that will remove all the auxin from tissues rapidly and with one extraction. Recently LINK, EGGERS, and MOULTON (10) described methods for the preparation of large, thoroughly homogeneous, desiccated samples from which sub-samples can be taken. Material thus prepared is highly suitable for auxin-extraction studies.

In this work an extensive study of the extraction and behavior of auxin from smut tumors of corn was undertaken. Also, for source material, corn stems, corn leaves, corn tassels, and four strains of the corn-smut fungus, *U. zea*, were used to some extent. This investigation was continued over a period of 3 years, during the course of which about 3000 pans of *Avena* were used in the tests. Most of the experiments have been repeated, some of them many times.

Material and methods

A strain of Northwestern Dent corn and four strains of *Ustilago zea* were obtained from Dr. E. C. Stakman of the University of Minnesota. The corn was grown in the greenhouse in garden soil, using 12-inch pots with four plants to each

¹ This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

pot. The tumors were produced by inoculating corn plants when they were about 1 foot tall. Injections of *U. zeae* were made into the region of the growing point with a hypodermic syringe. Monosporidial cultures of *U. zeae*, designated 10I₁, 10K₂, 10J₃, and 10J₄, were grown on a liquid 0.1 per cent bacto-tryptone-dextrose medium. To incite tumor production, except for certain solopathogenic strains of the microorganism, it is necessary to mix + and - strains at the time of inoculation. Strains 10I₁ and 10K₂ yield only local lesions but no tumors, when injected into the plants separately; when mixed in liquid culture just before inoculation, the two together are highly pathogenic and large tumors result, located principally on the stems and leaves. These two strains were used exclusively to incite the tumors for extraction. Strains 10J₃ and 10J₄, being solopathogenic, are each capable, when injected into the growing point, of inciting tumor formation. They were not, however, as virulent as a mixture of + and - strains. Studies were made on the yields of auxins from the mats of the fungus grown on liquid media, and in this work all four strains were tested. About 3-4 weeks after inoculation, tumors appeared and continued to be produced over a period of 2 weeks. In the autumn of 1940, samples of normal leaves and of tumors from stems and leaves were collected, frozen immediately by crushed dry-ice (CO₂) mixed with the methyl ester of ethylene (cellosolve). This gives a temperature of -80° C. and freezes the material rapidly. While frozen, the tissues were dried *in vacuo*. The dried material was ground in a Wiley micro-mill to pass a 60 mesh and then stored in an evacuated desiccator over phosphoric pentoxide. The powders proved to be stable and yielded active extracts. The auxin was assayed by means of the standard *Avena* test (22), using an initial decapitation period of 2 hours. Photographs were taken 100 minutes after application of agar blocks.

The *Avena* tests were made in a room especially constructed for the purpose. The temperature was held at 24° C., the relative humidity at 88 per cent, and a continuous supply of fresh air was brought in and circulated by fans. For the test plants a strain of Swedish Victory oats sold by Vaughan's Seed Company was used. This seed gave very satisfactory results. Germination was high, the coleoptiles grew at a uniform rate, the leaves pulled easily, and the sensitivity was high. The coleoptiles were calibrated at each test with standard agar blocks containing indoleacetic acid, the concentrations being 0.015 and 0.030 mg. per liter of agar. (The average curvature obtained with the former concentration was about 12°.)

Three extractants were used for the auxin extractions: an aqueous ethyl ether, dry ethyl ether, and water. The aqueous ether was freshly distilled for each experiment over wet FeSO₄ and CaO. Dry ether was obtained by treating it with potassium permanganate, drying with calcium chloride, and distilling over sodium; it was stored over sodium in the dark in a refrigerator. Prepared by either method it was always found to be peroxide free. When ether was used as the extractant

the extracts were evaporated to dryness in a water-bath at 50° C. and taken up in 2 cc. of melted 1.5 per cent agar. Serial agar dilutions were then poured into an 8×10×1.5 mm. mold. After hardening, the agar cast was cut into twelve equal blocks, which were applied to the *Avena* coleoptiles. In each dilution tested, twelve coleoptiles were used.

When water was the extractant, the extract was not taken to dryness. Instead, 1 cc. was pipetted from the sample to be tested and mixed directly with 1 cc. of melted 3 per cent agar. The dilutions and blocks were prepared as before. In all the extractions, care was taken not to overheat the preparations, and when the extracts were removed from a vessel the latter was washed two or three times with ether. Whenever duplicate samples were extracted and assayed together, they each gave the same number of units of auxin, within the limits of the variability of the *Avena* test itself.

The results of the tests have been expressed in arbitrary units for convenience of comparison where different materials and different methods of extraction were used. The arbitrary activity unit is defined as that amount of substance which—when put in 1 cc. of 1.5 per cent agar—causes 1° curvature after 100 minutes in the *Avena* test.

Experimental results

ETHER EXTRACTION OF FRESH MATERIAL

EXTRACTS OF ZEA MAYS.—During this work, fresh leaf samples were collected from corn of various ages and extracted in the usual way. Leaves from young corn plants were invariably found to be low in auxin. The highest auxin yields were obtained from leaves of plants coming into tassel. The tassels themselves were exceedingly high in auxin.

EXTRACTS OF CORN-SMUT TUMORS.—A 50-gm. sample of fresh tumor material from leaves and stems was collected in July, 1940, and placed in peroxide-free wet ether in the refrigerator at 4° C. for extraction. Care was taken to collect only young and medium tumors, older sporulating ones being avoided. The tumors obtained in the greenhouse were white or yellow and contained very little chlorophyll. The tissues, almost intact, were placed in 50 cc. of extractant per gram of fresh material. After 12 hours, the ether was removed from the sample, and the sample was washed with fresh ether and put back in new ether for a second extraction. The ether taken off was evaporated to dryness and tested by mixing the extract with 2 cc. of melted 1.5 per cent agar. Serial dilutions were made from this and assayed in the *Avena* test. The results of a series of five extractions are given in table 1. It will be observed from the table that in the first extraction the *Avena* curvatures are small and there is a lack of proportionality between the dilutions and the curvatures. This lack of proportionality was always observed in the first

extraction of the fresh tissues of tumors. In later extractions this peculiarity is less evident. It seems likely that in the first extraction there are masking substances present which prevent the expression of the auxin. The nature of these substances is unknown. Previous work (4, 8, 18, 17, 16, 21) indicates that substances with masking or inhibitory effects are frequently found.

Table 1 shows that after five successive ether extractions the sample was still yielding auxin, perhaps indicating continued auxin production over a period of time by the tumors. More data bearing on this point are presented later. Extractions of tumors from ears of corn collected in the field gave results in agreement with those just reported.

TABLE 1
ACTIVITY OF ETHER EXTRACTS OF FRESH SMUT TUMORS OF
MAIZE EXPRESSED IN AVERAGE CURVATURE PER
12 AVENA COLEOPTILES (DEGREES)

AMOUNT TESTED (GM.)*	TESTING DATES				
	7/12/40	7/19/40	7/22/40	7/25/40	8/16/40
50.....	7.1	14.3	13.9	19.2	12.9
25.....	7.8	16.8	11.2	16.9	10.0
12.5.....	8.7	17.3	3.6	12.6	3.7
6.8.....	9.2	15.8	1.5	6.8	0
3.4.....	6.8	8.8	0	3.1	
1.7.....	2.8	4.9		0	

* Number of grams of fresh material extracted represented by its portion of extract in 1 cc. of 1.5 per cent agar.

AUXIN CONTENT OF CORN LEAF SHEATH TUMORS COMPARED WITH NORMAL LEAF SHEATHS.—Fresh material was used and treated as already described. After 4 days of extraction the leaf sheath tumors gave forty-eight units of auxin as compared with three units for the normal sheaths. This relationship in which tumors are always higher in auxin than the normal tissues was found when the experiment was repeated and when repeated extractions of the same materials were made.

ETHER EXTRACTION OF FROZEN VACUUM-DRIED MATERIAL

AUXIN FROM SMUT TUMORS OF MAIZE: EXPERIMENTS WITH DRY ETHER.—Recently the development of the frozen vacuum-drying method for the treatment and storage of plant material has made it possible to compare different methods for the extraction of auxins. Using one finely ground sample of tumors of corn smut, studies were made of procedures for the extraction of auxin by drawing subsamples, treating them in various ways, and comparing the results of the different treatments.

With the dried material available for extraction, absolutely dry ether was prepared and used in making extractions. Dried material gave no activity when dry ether was the extractant. Independently of the present work, and using oven-dried material, THIMANN and SKOOG (20) have reported similar findings and concluded that water is necessary for auxin extraction of dry material. That auxin is soluble in dry ether has been demonstrated (10). Wet ether extracts of nodules of kidney bean, when dissolved in dry ether and then taken to dryness and assayed, showed the same activity as comparable wet ether extracts when taken up directly in agar. The conclusion is that water plays a part in the progressive liberation of auxin.

There was the possibility that the dried residue extracted by dry ether contained material which could be converted into auxin, if water were present. This was tested by a series of three extractions with dry ether, A, B, and C. Sample A

TABLE 2
ACTIVITY OF ETHER EXTRACTS OF 8 IDENTICAL 20-MG. SAMPLES
OF VACUUM-DRIED SMUT TUMORS FROM MAIZE

DATES OF TESTS	DAYS IN ETHER	UNITS OF AUXIN	DATES OF TESTS	DAYS IN ETHER	UNITS OF AUXIN
1/16/41.....	6	72	3/4/41.....	7	96
1/16/41.....	6	96	3/4/41.....	7	96
1/23/41.....	7	96	3/8/41.....	7	144
1/25/41.....	2	56	3/8/41.....	7	80

was tested without further treatment, B was treated with 1 cc. of water for 14 hours, and C with 1 cc. of water for 3 days. Extracts of A, B, and C were inactive in the *Avena* test, and it was concluded that dry ether does not remove any substance which can later be activated by the addition of water.

AUXIN FROM SMUT TUMORS OF MAIZE: EXPERIMENTS WITH WET ETHER.—Extractions of eight 20-mg. samples of frozen vacuum-dried material were made at different times with wet ether and are compared in table 2. The data are presented in terms of the arbitrary auxin units, defined earlier in the paper. Twenty cc. of ether was used for each sample and kept in the refrigerator at 4° C. The wet ether used in all the following experiments was prepared by shaking 15 cc. of dry ether, prepared according to the method already described, with 0.25 cc. of water. This amount of water saturated the ether. The procedure eliminated one source of variability in the extractions, since the dry ether used in all the extractions came from the same supply and contained the same amount of water. The ether used was in the ratio of 1000 cc. per gram of powder. The material remained in ether 7 days, except in two instances in which one extraction was for 2 days and the other 6 days. From four of the extracts 96 units were obtained (table 2); one gave

56 units, another 80, another 144, and another 72. When extracted for only 2 days, the auxin yield was 56 units. When the period of extraction in ether was the same, the number of units obtained was remarkably uniform. That time is a factor in the extraction process is shown in the following experiment.

Each of four 30-mg. samples of tumor powder, prepared according to the freezing desiccation method, was placed in 20 cc. of wet ether. Sample 1 was extracted one day; sample 2, two days; sample 3, three days; sample 4, seven days. The experiment was arranged so that the extractions were completed simultaneously, and all were assayed simultaneously. For sample 1 the yield was 44 units; for sample 2 it was 80 units; for sample 3 it was 120 units; and for sample 4 it was 320 units. There is good proportionality between time in wet ether and yield of auxin.

Tests to determine whether the amount of extractant used was a limiting factor showed that different quantities of ether resulted in practically the same number

TABLE 3
AUXIN FROM 20 MG. OF VACUUM-DRIED TUMORS FROM MAIZE SAMPLE
EXTRACTED WITH SUCCESSIVE 20-CC. VOLUMES OF ETHER

DATES OF TESTS	DAYS IN ETHER	UNITS OF AUXIN	DATES OF TESTS	DAYS IN ETHER	UNITS OF AUXIN
3/31/41.....	3	48	5/18/41.....	10	98
4/18/41.....	18	384	5/29/41.....	11	104
4/23/41.....	5	112	6/7/41.....	10	96
5/8/41.....	15	176			
Total to date.....				72	1018

of units of auxin. This corroborates the former observation that auxin is gradually liberated from dried material in the presence of water.

Experiments with repeated extractions of material with wet ether give further support to this observation and are of interest in comparison with the repeated extractions of fresh material discussed earlier. From one series of repeated extractions (table 3) it is evident that the fresh and the dried material on extraction gave similar results. When extracted, both yielded auxin over a long period. Here again the yield of auxin is a function of the extraction time. The fresh material differed greatly from the dry powder, however, in that dry material yielded about 100 times as much auxin as the fresh when the same number of grams of each, on a fresh-weight basis, were used in making the extractions.

In the repeated extractions just described, either dry ether or wet ether was used alone. In another experiment, two 30-mg. samples, A and B, were first extracted with wet ether for 14 days and tested, the residues dried by washing with dry ether, followed by a second extraction in dry ether for 8 days, after which these extracts were tested while the residues were placed in wet ether for a third extrac-

tion. In this third extraction, sample A was in wet ether for 5 days and B for 12 hours. The first extraction with wet ether gave for A and B, 320 and 277 units, respectively; the second extraction with dry ether gave 0 units in each case; the final wet ether extracts were active. From sample A after 5 days, 138 units were obtained, while the yield from B after 12 hours was 40 units. This is further evidence of the part played by water and the importance of the time factor in auxin liberation.

The Soxhlet method of extraction with wet ether was tried with the possibility that auxin in the unbound form might be rapidly removed in this way. For the

TABLE 4

ACTIVITY UNITS OF EXTRACTS OF TWO 20-MG. SAMPLES OF POWDER SOXHLETIZED FOR 18 AND 47 HOURS, RESPECTIVELY. FOLLOWING THE 18-HOUR SOXHLETIZATION THIS SAMPLE WAS EXTRACTED BY STANDING IN SUCCESSIVE VOLUMES OF ETHER IN ICEBOX

DATE OF EXTRACTION	DAYS IN ETHER	SOXHLETIZED 18 HOURS	SOXHLETIZED 47 HOURS
3/21/41.....		288 (stand- ing)	384
3/22/41.....	1	14	
3/28/41.....	6	28	
4/12/41.....	16	60	
4/22/41.....	10	32	
5/7/41.....	15	28	
5/29/41.....	21	12	
6/7/41.....	10	9	
Total.....	79	471	

tumor material, this does not seem to be the case; almost all the auxin is bound. With short Soxhlet extractions of 3, 6, and 10 hours very low yields were obtained, scarcely greater than if the material had been merely standing in ether for the same periods. Table 4 summarizes the data from two Soxhlet runs, one of 18 hours' duration and the other of 47 hours. It is observed that there is no proportionality between the length of time the material was Soxhletized and the yield of auxin. Other materials extracted in the Soxhlet gave similar results, but no explanation for this behavior is offered.

After the 18-hour Soxhletization, the powder was re-extracted seven times with wet ether, over a period of 79 days. The total auxin yield, including the Soxhletization, was 471 units, as compared with an expected yield of about 1000 units for repeated extractions of the material when left standing in the refrigerator in wet ether. Considerable auxin was lost when the Soxhlet method was used.

Two Soxhlet extractions with dry ether, A and B, were followed by extraction

of A with wet ether and B with water (table 5). There was the possibility that when fats and other substances were removed by dry ether the process of liberation of auxin would be more complete and rapid. After this Soxhletization by dry ether, the material gave similar results in subsequent extractions to powders subjected to repeated wet ether extraction alone, although auxin yields were considerably lower after the Soxhlet procedure.

AUXIN FROM SMUT TUMORS OF MAIZE: WATER EXTRACTION. —A 20-mg. sample of tumor powder was extracted for 2 days in dry ether to which 0.5 cc. of water had been added. By separating the ether fraction from the water fraction and testing each separately for auxin content, it was determined that the water fraction was many times more active than the ether fraction. It seems that the extractable

TABLE 5
ACTIVITY UNITS OF EXTRACTS OF SAMPLES A AND B, EACH
SOXHLETIZED WITH DRY ETHER, A THEN EXTRACTED
BY STANDING IN WET ETHER 8 DAYS AND B IN WATER
8 DAYS. CONTROLS FOR A AND B EXTRACTED BY
STANDING IN WET ETHER AND WATER 8 DAYS IN ICE-
BOX, RESPECTIVELY

A WET ETHER		B WATER	
SOXHLETIZED, THEN IN ETHER 8 DAYS	CONTROL, IN ETHER 8 DAYS	SOXHLETIZED, THEN IN WATER 8 DAYS	CONTROL, IN WATER 8 DAYS
60	192	2176	2304

auxins are highly soluble in water and not very soluble in ether, indicating that the partition coefficient between ether and water is low for these auxins.

These findings suggested some studies on water extraction of the tumor powder. Some of the experiments are similar to those carried out with wet ether. They were devised so that the efficiency of ether and of water as extractants could be compared. Table 6 gives the results of a series of repeated extractions. Ten mg. of powder was put into 2 cc. of water for this series, the ratio of extractant to powder thus being 200 to 1. Experience had shown that more than enough water was present for the amount of powder to be extracted. In making the agar blocks for the *Avena* test, 1 cc. of water extract was put into 1 cc. of 3 per cent agar and dilutions made from this. The powder was put into previously unused distilled water for each subsequent extraction. All extractions were carried out in the refrigerator at about 4° C. Most of the auxin was liberated in the first three extractions. For example, 3008 units (table 8) were obtained from three extractions which

covered 22 days, and only 198 units were obtained in the next three extractions throughout a 33-day period. Comparable repeated extractions by ether and by water were made, and it was found that when water was the extractant the yield of auxin was greater than when ether was the extractant. The total number of units of auxin obtained by exhaustive extraction with water was 3000-3500, while with wet ether only 1000-1500 units were obtained. Greater yields of auxin were obtained in a given time when the material was extracted with water than when ether was the extractant. As an example, in one experiment the auxin yield was

TABLE 6

AUXIN FROM 10 MG. OF VACUUM-DRIED SMUT TUMORS OF MAIZE.
SUCCESSIVE WATER EXTRACTIONS EACH OF 2 CC.

DATES OF TESTS	DAYS IN WATER	UNITS OF AUXIN	DATES OF TESTS	DAYS IN WATER	UNITS OF AUXIN
4/21/41.....	9	1280	5/7/41.....	12	104
4/22/41.....	10	1152	5/18/41.....	11	88
4/25/41.....	3	576	5/28/41.....	10	6
Total.....				55	3206

TABLE 7

AUXIN FROM 5 MG. OF VACUUM-DRIED SMUT TUMORS
OF MAIZE. SUCCESSIVE WATER EXTRACT-
TION EACH OF 2 CC.

TIME IN WATER	UNITS OF AUXIN	TIME IN WATER	UNITS OF AUXIN
$\frac{1}{2}$ hour.....	288	$\frac{1}{2}$ hour.....	0
$\frac{1}{2}$ hour.....	32	5 days.....	128

256 units in 2 hours, 512 in 24 hours, and 1536 in 7 days. In sharp contrast is the fact that wet ether gave on the average only 96 units in 7 days.

Another proof that auxin does not all come out at once when water is the extractant was provided by the following experiment. In this case 5 mg. of powder was put into 2 cc. of water for $\frac{1}{2}$ hour. The water was removed from the sample and the latter again put into 2 cc. of water. The material was treated thus with water three times for $\frac{1}{2}$ hour each time. Each 2 cc. of water removed was tested for auxins. Auxin was obtained in the first two extractions (table 7) but not in the third. The powder, apparently exhausted of auxin, was extracted again for 5 days. Tests showed 128 units present at the end of that time. Liberation of auxin must have occurred during the 5-day interval. The experiments were carried out at

2° C. and there was no growth of microörganisms. The experiment was repeated with similar results.

In the experiment (table 7) in which the powder was Soxhletized with dry ether and then extracted 8 days with water, it is noted that 2176 units were recovered, compared with 2304 units from control material not Soxhletized. Thus, removing fats and other substances with dry ether subsequent to water extraction did not aid the process of extraction.

Using water extracts, the effect of heat on the auxin extracted by water from smut tumors was studied. Ten mg. of powder was extracted in 4 cc. of water for 20 hours. At the end of this period, the water was divided into four aliquots. Sample 1 was placed in the icebox at 1.7° C., vials containing samples 2, 3, and 4 were

TABLE 8
EFFECT OF TEMPERATURE ON AUXIN STABILITY;
EXPOSURE 2 HOURS IN WATER

SAMPLE	TEMPERATURE (° C.)	UNITS OF AUXIN	SAMPLE	TEMPERATURE (° C.)	UNITS OF AUXIN
1.....	1.7	64	3.....	60	72
2.....	40	64	4.....	100	44

placed in hot-water baths at 40°, 60°, and 100° C., respectively, for 2 hours. The activity of the several extracts was then tested (table 8). The heat did not change the amount of auxin in the samples under the conditions of the experiment, except at 100° C.

ETHER EXTRACTION OF FOUR STRAINS OF *USTILAGO ZEAE*

Four strains of *U. zeae* were grown in a liquid bacto-tryptone medium. After 2 months the mats of the fungus were removed from the surface of the culture medium and placed in wet ether for extraction. The ether was then decanted off, the mats washed several times with fresh ether, and returned to fresh ether for a second extraction. The ether was taken to dryness after each of the two extractions, and the extracts were tested for auxins. It was found that almost all the auxins were removed in the first 24-hour extraction period. When third extractions were made no auxins were obtained. This is in contrast to green tissues which continue to yield auxins, even after ten or more extractions.

For another series of experiments the mats of the fungus were frozen and vacuum dried and the material then ground to a fine powder. The powders were treated with wet ether. Analyses showed the total auxin obtained to be greater than the total obtained by treating comparable fresh mats with ether. Experience has shown that no auxin is obtained when dry ether is used in the extraction of the dry powders.

Strains 10J₃, 10J₄, 10I₁, and 10K₂ were grown on two different media in order to determine whether or not different amounts of auxin could be obtained from the various strains grown on an organic bacto-tryptone medium, and also whether auxin could be obtained from mats grown on a synthetic medium containing inorganic salts and dextrose but no proteins or amino acids. The four strains were grown for two different periods (2 months and 9 months) on each of the two media. The mats were then frozen and vacuum dried. Ten-mg. samples of the strains of the fungus grown on the organic bacto-tryptone medium were extracted with 20 cc. of wet ether. Eighty-mg. samples of the fungus grown on the synthetic medium were extracted at the same time. Table 9 shows that extracts of the four

TABLE 9
ACTIVITY OF ETHER EXTRACTS FROM FOUR STRAINS OF USTILAGO ZEAE. EACH STRAIN GROWN ON ORGANIC BACTO-TRYPTONE MEDIUM OR ON SYNTHETIC MEDIUM FREE OF PROTEINS AND AMINO ACIDS

STRAIN	BACTO-TRYPTONE MEDIUM		SYNTHETIC MEDIUM
	TWO MONTHS ON MEDIUM, UNITS OF AUXIN FROM 10-MG. SAMPLE	NINE MONTHS ON MEDIUM, UNITS OF AUXIN FROM 10-MG. SAMPLE	TWO MONTHS ON MEDIUM, UNITS OF AUXIN FROM 80-MG. SAMPLE
10J ₃	42	200	23
10J ₄	10	100	32
10I ₁	8	100	4
10K ₂	4	60	8

strains differ in the amount of auxin which can be obtained by extracting the powders. The extracts from the solopathogenic forms, except in one case, show a higher auxin content than the extracts from the non-solopathogenic ones. It can also be seen that auxin can be obtained from the fungus grown on the synthetic medium free of proteins and amino acids.

The powders from the fungus grown on the organic medium, however, yield about eight times as much auxin as the same amounts of powders from fungus grown on the synthetic medium. In the experiments just described the mats alone were tested for auxins. The next step was to test the liquid media that had supported the fungus.

Forty cc. of both types of liquid medium that had supported the mats of the fungus were dried separately *in vacuo*. The residues were dissolved in 2 cc. of water and mixed with 2 cc. of melted 3 per cent agar. The agar blocks for the *Avena* test were cast from this mixture. From both types of medium very active

extracts were obtained. They were about equally auxinic. The source of the auxin is not known. The organisms may synthesize auxin intracellularly, followed by its diffusion from the cells into the medium, or the cells may produce substances which diffuse into the medium and convert materials in the nutrient to auxin. The fungus on the bacto-tryptone may convert the tryptophane or other organic compounds (either intercellularly or intracellularly) into auxin. In the case of the fungus grown on the synthetic medium in which dextrose was the only organic substance, it seems likely that the auxins were produced intracellularly. The function of the auxins in the metabolism of the fungus, if they have any function, is not known. Perhaps they are katabolic products.

Discussion

Total auxin yields from frozen vacuum-dried powders from ground tumors of corn smut, or leaves, or the fungus *Ustilago zeae*, were higher than from the same objects in the fresh state. The same findings have been reported for kidney bean roots (10), for legume nodules, and for crown gall of tomato. THIMANN and SKOOG (20) noted that material which had been dried by heat gave a decreased yield of auxin, perhaps due to heating.

The results of this work, and of the investigations just cited, indicate that water plays some role in the liberation of auxin from plant tissues. That its action is hydrolytic has been suggested (10). SKOOG and THIMANN (15) have released bound auxin with proteolytic enzymes. Apparently any free auxin present in the living tissues is destroyed during the freezing and dehydrating process, or is fixed during these processes, since none is removed with dry ether—in which it is soluble.

The auxin from smut tumors is highly soluble in water, much more so than in ether, and comparisons of exhaustive extractions made with the two extractants indicate that the process is more rapidly and completely carried out with water. During the slow ether extraction, perhaps much auxin is destroyed by peroxides and otherwise, explaining the higher total yields from the more rapid water extraction.

All the auxin is obtained from *U. zeae* preparations in a single extraction with wet ether. This is in contrast to green tissues requiring ten or more extractions to obtain the same results.

Strains of *U. zeae* produced auxin when grown either on a bacto-tryptone medium or on a synthetic medium without proteins of amino acids. Auxins have been extracted from microorganisms grown on media containing amino acids or proteins (14, 7, 19, 1, 9, 2, 3). BURKHOLDER (2) demonstrated that *Aerobacter aerogenes* and *Escherichia coli* grown on glycerol-mineral salts-agar, in which the sole

source of nitrogen was either KNO_3 or NH_4Cl , produced auxin. GEORGI and BEGUIN (3) reported the elaboration of growth substances by four species of *Rhizobium* and *Bacillus radiobacter* when the culture medium contained tryptophane. They found, however, that when mannitol was the source of carbon and KNO_3 the sole source of nitrogen, no active substances were produced in the medium.

Solopathogenic strains of *U. zae* were found, with one exception, to produce more auxin than non-solopathogenic strains. This ability of the solopathogenic strains may be correlated with the capacity of the former to excite tumors in corn. LOCKE, RIKER, and DUGGAR (13) reported that for *Bacillus radiobacter* no consistent difference in the production of growth substances was observed between the virulent and the attenuated cultures. GEORGI and BEGUIN tested virulent and attenuated strains of *Rhizobium* and also found no correlation between pathogenicity and auxin yield.

HAAGEN-SMIT, LEECH, and BERGEN (6) have isolated crystalline indoleacetic acid from extracts of corn. This is the first time that this acid has been isolated from higher plants. These workers conclude that since the amount of the isolated acid was many times greater than the auxin amount indicated by the normal extraction process, it represented a large part of the auxin present in bound (precursor) form. Thus it is likely that the auxin extracts of corn contained indoleacetic acid and probably other active substances.

Differences in auxin content exist between smut tumors and similar regions of healthy tissues, the tumors being the more auxinic. LINK has found that in all cases where tumor material has been compared with healthy, the former was the more active. In this connection LINK and EGGERS (11) have recently reported on legume nodules and crown gall of tomato.

The higher activity of the tumors could be due to (a) the metabolic activity of the pathogen, or (b) the activity of the tumor tissues themselves in response to the injury of the host cells by the parasite. It is clear that auxin may be one of the causes of the pathological condition manifested by tumor production.

During the last 5 years many reports have appeared concerning the role of auxins in the physiology of plants. The only conclusion that can be reached from a review of the literature is that there is no simple relationship of growth-regulating substances to metabolism; apparently they play various roles, both direct and indirect. In general, auxins are found in highest concentrations in regions of the plant that are young and actively growing, that is, regions where the metabolic rate is high. It is possible that the presence of large amounts of auxins in the tumors studied is correlated with the increased and abnormal metabolism of the infected tissues.

Summary

1. The extraction of auxin by ether and by water from smut tumors of corn and other materials, frozen and vacuum dried, was studied. For a given weight of smut tumors, water extraction yields the greater amount of auxin. Dry ether extracts are not active. Water is necessary for the liberation of auxin from the tissues. Its action may be hydrolytic.

2. Smut tumors of corn yield auxin slowly with either water or ether extraction. The auxin is almost completely removed from the fungus, *Ustilago zaeae*, in one ether extraction.

3. Smut tumors from corn leaves or stems yield more auxin than healthy leaves or stems.

4. Strains of *U. zaeae* grown on a synthetic medium containing neither proteins nor amino acids are able to produce auxin.

5. Extracts of both types of medium upon which *U. zaeae* had grown for 2 months contained much auxin and practically the same amount in each case.

6. The pathogenicity of strains of *U. zaeae* seems to be correlated with their ability to produce auxin in a bacto-tryptone or a synthetic medium.

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